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(54) Title: READILY ABSORBABLE PHYTOSTEROLS TO TREAT HYPERCHOLESTERROLEMIA

(57) Abstract

A process of preparing sterols in a form that is easily absorbed through the digestive tract, and methods of treating cholesterolemia by administering a composition which includes at least one sterol selected from a group consisting of β–sitosterol, stigmasterol and campasterol and saturated sterols corresponding thereto.

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READILY ABSORBABLE PHYTOSTEROLS TO TREAT HYPERCHOLESTEROLEMIA

Background of the Invention

Phytosterols are components of plants and grains and are contained in small amounts in their cells. About 100 different phytosterols have been isolated from plants. The most abundant by far are (in order) sitosterol, campesterol and stigmasterol. They are structurally very similar to cholesterol except they are alkylated at the 24-position in the side chain.

Phytosterols are natural components of the diet and are consumed in amounts of 100-500 mg/day with US consumption being generally low. (Weirauch, J.L., Gardner, J.M. 1978. Sterol content of foods of plant origin. *J. Am. Diet. Assoc.* **73**:39-47) Reportedly, phytosterols themselves are absorbed in relatively small amounts. (Grundy, S. M. and H. Y. I. Mok. 1977. Determination of cholesterol absorption in man by intestinal perfusion. *Journal of Lipid Research* **18**:263-271)

The effect of phytosterols on cholesterol absorption has been studied in humans using intestinal intubation where cholesterol is infused and its disappearance is measured in the presence or absence of phytosterols. Using this technique and giving sitosterol in a micellar form (dissolved in monoglyceride), the amount of cholesterol absorbed reportedly was reduced, declining by 79% of the mass of sitosterol infused. (Martin et al., *Lancet*, 2:933-936 (1986) In 2 out of 5 sitosterol infusions cholesterol absorption was reduced to zero indicating an extraordinary potential for

efficacy. In 8 clinical trials low density lipoprotein (LDL) cholesterol was lowered 7-33% by sitosterol or its 5-ß-reduced metabolite sitostanol. (Ling, W. H. and P. J. H. Jones. 1995. Dietary phytosterols: A review of metabolism, benefits and side effects. *Life Sciences* **57**:195-206) Sitostanol reportedly is more effective than sitosterol for cholesterol lowering in animals (Sugano, M., Morioka, H., Ikeda, I. 1977. A comparison of hypocholesterolemic activity of beta-sitosterol and beta-sitostanol in rats. *J. Nutr.* **107**:2011-2019) and has nearly no absorption from the intestine. (Hassan, A.S.,Rampone, A.J. 1979. Intestinal absorption and lymphatic transport of cholesterol and beta-sitostanol in the rat. *J. Lipid Res.* **20**:646-653) Sitostanol given primarily as the oleate ester in margarine reduced serum LDL cholesterol by 14% in a random population sample of 153 individuals with moderate hypercholesterolemia (mean 237 mg/dl) and moderate dietary cholesterol intake of 308-340 mg/day. (Miettinen, T. A., P. Puska, H. Gylling, H. Vanhanen, and E. Vartiainen. 1995. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *New England Journal of Medicine* **333**:1308-1312) In the subgroup receiving 2.6 g/day of sitostanol oleate total plasma cholesterol declined from 235 mg/dl (at the upper end of the NCEP "borderline high" classification) to 210 mg/dl (near "ideal") while LDL cholesterol declined 16%.

Miettinen et al. *New England J. Med.*, November 16, 1995, pp 1308-1351 discloses that fatty acid esters of phytosterols reduce cholesterol.

Although 1.8-2.6 g/day sitostanol oleate solubilized in rapeseed oil margarine was highly effective in reducing serum cholesterol, in another

trial, 3.0 g/day of unesterified sitostanol suspended (not dissolved) in a small amount of oil was ineffective. (Denke, M. A. 1995. Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. *Am. Journ. of Clinical Nutrition* **61**:392-396)

Phytosterols may be given orally as the free sterols in aqueous suspension or as dry powders. However, phytosterols are insoluble in water and poorly soluble in oil and it may take several days to achieve final equilibrium solubility when sitosterol crystals are added to aqueous bile salt micelles. (Armstrong, M. J. and M. C. Carey. 1987.Thermodynamic and molecular determinants of sterol solubilities in bile salt micelles. *Journal of Lipid Research* **28**:1144-1155)

Summary of the Invention

The present invention relates to sterols and hydrogenated sterols in a form that is easily absorbed through the digestive tract. The invention also generally features a method of treating cholesterolemia. The composition and method for treating cholesterolemia comprises of at least one phytosterol selected from a group consisting of ß-sitosterol, stigmasterol and campasterol and saturated phytosterols corresponding thereto, said sterol being dissolved or dispersed in a solubilizing macromolecule. Dispersion is particularly important because, surprisingly, the dispersed material is absorbed through the intestine at a substantial concentration, enhancing anti-cholesterolemic effect without the need to use substantial amounts of fatty substances as carriers.

Particular useful solubilizing macromolecules include phospholipids and starch, modified starch, alphalized starch, dextrin, sodium starch phosphate, glucose, lactose, monosaccharides, disaccharides, polysaccharides hydroxypropyl cellulose, methyl cellulose, and lecithin. Saturated (e.g., hydrogenated sterols) or unsaturated sterols may be used. Particularly useful sterols include the ß-sitosterol, campasterol, and stigmasterol or the hydrogenated form thereof. The therapeutic may be prepared from a solid residue remaining after removal of water or other solvents from a solution or suspension of said sterol and the carrier or diluent. Dispersions of the sterols can also be obtained by milling them with solid carriers. Typically, the sterol has a particle size of 1 - 100 micron. These formulations are particularly useful as oral pharmaceutical compositions comprising an effective amount of the sterol and a pharmaceutically acceptable carrier or diluent.

Phytosterols are not water-soluble and, if they are not absorbed, they may be excreted after ingestion with little or no effect to lower cholesterol. The invention enhances bioavailability of phytosterols by enhancing absorption in the intestine.

The invention also avoids discomfort and other problems associated with oral administration of phyosterols -- e.g,, pure phytosterols pressed into one-gram tablets can create stomach disorders. We concluded that these tablets cannot be readily digested and absorbed and therefore create a discomfort. Unmixed sitosterol powder may appear in stool samples from patients undergoing cholesterol turnover studies where sitosterol was given as a stool marker.

According to the invention, sitostanol (for example) is delivered in a more soluble form without using oil or margarine as a vehicle avoiding the substantial disadvantage of administering oil to a patient in need of cholesterol reduction -- giving 3 g/day of sitostanol oleate in oil requires about 30 g oil with 270 calories.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiment and from the claims.

Description of the Preferred Embodiments

We have prepared dispersions of phytosterols and the hydrogenated phytosterols in dispersions of liquids and solids.

Suitable sources of sterols include soybeans, wood, and apples. The sterols may be obtained from these sources by known techniques, e.g., by extraction and recrystallization Liposomes containing the sterols and hydrogenated sterols may be prepared by techniques generally described below.

Typical dosages according to the invention are from 10-500 mg/75kg patient. This dosage may be formulated in a powder and dispersed in a polymer such as starch as described below. The dispersion is inserted into a standard soft gel capsule or hard capsule.

Various techniques are known to test the dosage in animals and humans.

Examples of Preparation Procedures

Preparation of Hydrogenated Phytosterols

Phytosterols from soybeans containing sitosterol campesterol and stigmasterol (30 gram) was dissolved in 400 ml of ethylacetate and poured into a 600 ml stainless steel pressure vessel. Two grams of palladium on carbon (10% dispersion) was added. The pressure vessel was charged with hydrogen to a pressure of 1000 psi and magnetically stirred. After 2 hours no additional pressure drop was observed. After 24 hours the pressure was released and the content of the vessel was filtered to remove the catalyst. The solvent was evaporated on a rotary evaporator. The dry product was re-crystallized from hot ethanol two times. A sample dissolved in $CDCl_3$ analyzed by NMR showed the absence of double bonds.

Preparation of Dispersions with Phytosterols, Hydrogenated Phytosterols.

Dispersions in Lecithin (Phospholipids)

Five gram of soybean sterol was added to 20g of Lecithin. A high-speed stirrer was used to disperse the sterols. The stirrer with sheer action consisting of an outer cylinder and stirring blades rotating inside the cylinder was used. The rotating blades are projecting and pressing the granules against the inside of the cylinder and grind them to smaller particles. The size of the particles can be varied by the duration of the stirring and by increasing or decreasing the rotation of the blades. Particle sizes of 1 - 50 microns were obtained. The particle sizes of the dispersion were determined by filtration with a Millipore filter. The dispersion was passed through the filter of defined pore sizes of 20 - 50 micron by vacuum suction. All of the dispersed particles passed through the filter indicating that the dispersion contained particle size of less than 50 microns.

Dispersions in solid carriers

The sterol is dissolved or dispersed by heating in water. Water soluble or dispersible polymer or polymers are added like starch or modified starch includes natural starch obtained from corn, potato or arrowroot, alphalized starch, dextrin. Water soluble starch or cellulose derivatives such as esterified starch (sodium starch phosphate), hydroxypropyl cellulose, methyl cellulose, and the like can also be used.

A typical preparation example:

In 300 ml of water 6 gram of cornstarch was dispersed with a high-speed mixer, 0.6g of soybean sterol was added while stirring. The mixtures were dispersed at temperatures of 50 C for 30 minutes. Afterwards the water was removed with a rotary evaporator. The obtained cake was pulverized in a ball mill for 5 hours. To test the particle sizes of the solid dispersion 0.5 gram of the mixture was dispersed in 100 ml of water with a high-speed mixture. The suspension was filtered through a Millipore filter of defined pore size of 20 - 50 micron. No residue was collected on the filter indicating particle sizes were obtained of less then 50 micron.

The complete effect of phytosterols on serum cholesterol may require several months for full expression. See, Miettinen et al., *New Eng. J. Med.* 333:1308-1312 (1995), and Goodman et al. *J. Lipid Res.* 21:699-713 (1980) and clinical trials with respect to cholesterol lowering are designed accordingly. The total body cholesterol burden is approximately 72 g and compartmental turnover times may be a matter of weeks or even several months.

Inhibition of cholesterol absorption can be determined over a short time period. However, very few clinical trials have actually measured cholesterol absorption because it has required the use of radioactive isotopes and stool collection or gastrointestinal intubation. Non-radioactive cholesterol tracer molecules may be labeled with deuterium and detected by mass spectrometry to measure cholesterol absorption directly, (Lutjohann, D., C. O. Meese,J. R. Crouse,III, and K. von Bergmann. 1993. Evaluation of deuterated cholesterol and deuterated sitostanol (provided by Medical Isotopes, Inc.) for measurement of cholesterol absorption in humans. *Journal of Lipid Research* **34**:1039-1046) and that technique can be used to measure the effect of the invention on absorption.

What is claimed is:

CLAIMS

1. A method of treating cholesterolemia by administering a composition which comprises at least one phytosterol selected from a group consisting of ß-sitosterol, stigmasterol and campasterol and saturated phytosterols corresponding thereto, said sterol being dissolved or dispersed in a solubilizing macromolecule.

2. A method according to claim 1 wherein the phytosterol is a saturated phytosterol.

3. A method according to claim 1 wherein the solubilizing macromolecule is a phospholipid.

4. A method according to claim 1 in which the solubilizing polymer is a carrier or diluent selected from the group consisting of starch, modified starch, alphalized starch, dextrin, sodium starch phosphate, glucose, lactose, monosaccharides, disaccharides, polysaccharides hydroxypropyl cellulose, methyl cellulose, phospholipids and lecithin.

5. The method of claim 3 wherein the phytosterol is part of a solid residue remaining after removal of water or other solvents from a solution or suspension of said sterol and the carrier or diluent.

6. A method according to claim 1 wherein the sterol has a particle size of 1 - 100 micron.

7. An oral pharmaceutical composition comprising an effective amount of a saturated sterol and a pharmaceutically acceptable carrier or diluent.

8. A composition according to claim 7 wherein the sterol has a particle size of 1 - 100 micron.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/02578

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/56
US CL :514/182
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/182

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

MERCK INDEX

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database BIOSIS on STN, No. BA71:5097, 1981:135105, TABATA, T. et al., "Hypocholesterolemic activity of Phyto Sterol 2.", Abstract to Yakugaku Zasshi, 1980, 100, 546-52,	7 and 8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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INTERNATIONAL SEARCH REPORT

International application No.
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Electronic data bases consulted (Name of data base and where practicable terms used):

REGISTRY, MEDLINE, CAPLUS, BIOSIS, EMBASEM, WPIDS search terms: cholesterolem?, phytosterol or stigmasterol or campasterol or sitosterol, starch or dextrrin or sodium starch phosphate or glucose or lactose or monosaccharide or disaccharide or polysaccharide hydroxylpropyl cellulose or metyl cellulose, phospholipid and lecithin.